CELL-MEDIATED IMMUNITY IN MEASLES

THESIS

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C E R T I F I C A T E

This is to certify that the work entitled "CELL MEDIATED IMMUNITY IN MEASLES" has been conducted by Dinesh Kumar in the department of paediatrics, M.L.B. Medical College, Jhansi. He has put in the necessary stay in the department according to university regulations.

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C E R T I F I C A T E

This is to certify that work entitled "CELL MEDIATED IMMUNITY IN MEASLES" has been conducted by Dinesh Kumar under my guidance and supervision in the department of paediatrics, M.L.B. Medical College, Jhansi.

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This is to certify that the work in connection with thesis on "CELL MEDIATED IMMUNITY IN MEASLES" was conducted by Dinesh Kumar in the department of Pathology under my guidance and supervision. The techniques incorperated in the thesis were under taken by the candidate himself and observations recorded have been periodically checked by me.

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C O N T E N T S

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INTRODUCTION

INTRODUCTION

Measles is a highly infectious disease of varying severity occuring in human beings. It is one of the most common infectious disease of early childhood, endemic in many areas of the world, with recurrent epidemics, showing a characterstic epidemiological pattern based on the ratio of immunes and susceptibles in the population.

It is well known that extensive immunosuppression exists during acute measles (Coovadia et al., 1978). However controversies still exists regarding the mechanism of immunosuppression especially in young children. Possible hypothesis have been advanced in support of immunosuppression, Whittle, H.C. and Dossetor, J. (1978) have suggested that the deplition of T cells, an inhibitor of lymphocyte proliferation in the serum and a possible defect in antigen processing interacts to depress cell mediated immunity in measles.

The study of Whittle H.C. and Bradley Moore (1973) have shown that measles causes a temporary suppression of the skin reaction to PPD, Candida and Streptococcal antigens. However, when the expression of delayed hypersensitivity was suppressed, the patient could still be sensitized normally to Dinitrochlorobenzene (DNCB) and their lymphocyte responded to stimulation with PHA.

It is now well established that cell mediated immunity is important not only for recovery from measles, but also for resistance to other bacterial and viral infections. A deficit in cellular immunity can favour the emergence of complications in measles. Tuberculosis and moniliasis infections normally controlled by cell mediated immune responses, are known to follow measles (Bech 1962, Smythe et al., 1971). Pyogenic infections, which are usually limited by humoral immune responses, are also freequent and severe in children with measles. Morbidity due to measles shows two distinct phases. The children may develop complications in the acute stage of measles or during the subsequent period. Prolonged morbidity due to secondary infection is freequent especially in malnourished children, this has been attributed to immunosuppression. Many speculate that this phenomenon is due, at least in part, to the impairement in cellular immunity observed in malnourished children, especially during measles. Schiefele and Forbes (1973) have postulated that measles is severe in malnourished children owing to defect in the formation of activated lymphocytes. In support of this argument children with oedematus malnutrition have been shown to be immunosuppressed. They have lymphopenia

(Smythe et al., 1971), a deficiency of T cells (Schopfer and Douglas, 1976) and fail to react to many common antigens. However Whittle has demonstrated that in malnourished children, although peripheral blood mononuclear cells (PBM) support a higher replication of measles virus, their cellular immunity does not seem to differ from that in well nourished children (Dossettor J. et al., 1977).

Studies by Bhaskaran P. et al. (1984) have indicated that there is an equal degree of immunosuppression in both malnourished as well as well nourished children. However their subsequent studies (1986) have revealed a significant depression in circulating T lymphocytes, in severely malnourished children compared to those of other nutritional grades. Ron Dagon et al. (1987) have shown that there was a more impressive depression in T cell count than the B cell in malnourished children with acute measles, both T and B cell counts have been shown to be increased in convalescent phase of the illness.

Immunological studies in measles indicate that the profound immunosuppression during the first few days of the rash has been shown to affect chiefly T and B cell subpopulations, with less severe effects on C_3 and T cell function assessed by PHA transformation of lymphocytes (Coovadia et al., 1978). Elements of immunosuppression

persists upto 3 weeks and then there is return towards the normal at 6th week in uncomplicated cases. Both T and B cells have been shown to be infected in disease process and they also support replication of virus (Pelton Winsome et al., 1982). Per Arneborn and Gunnel Biberfeld (1984) noticed T lymphocytopenia but no change in the ratio between T lymphocytes of helper and suppressor/ cytotoxic cell phenotypes.

Controversies, however still exists regarding the B cell count which have been shown to be depressed (Coovadia et al., 1978). While others have noticed no significant change in the B cell subpopulation (Whittle H.C., Dossetor 1978 and Ron Dagon et al., 1987).

There are many studies highlighting T cell and B cell function. But no other work have been performed to our knowledge comprising T and B cell function in malnourished children and nourished children suffering from measles along with skin reaction to Dinitrochlorobenzene (DNCB). In the light of above observations the present study was undertaken to evaluate the immunological responses in cases of measles during the first week of illness.

AIMS AND OBJECTIVES

1. To assess the immunological status in patients of measles by T cell count, B cell count and skin reactivity by DNCB test.

2. To evaluate the co-relation between lymphocyte and DNCB skin test.

3. To compare the cell mediated immunity in malnourished and well nourished children suffering from measles.

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REVIEW OF LITERATURE

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Measles in one of the important exanthamous viral infections of childhood. The disease has been defined as "a eruptive fever caused by a specific virus and clinically characterized by fever and catarrhal symptoms followed by typical rash."

Measles is a "pediatric priority" in the developing countries (Morley, D. 1973). The outcome of the disease depends upon the cell mediated immune system of the host (Burnet, F.M. 1968).

NOMENCLATURE AND HISTORY

There is some doubt about the origin of the name measles most probably it comes from the Latin term misellus or misella itself a diminutive of the Latin miser, meaning miserable. It was John Gaddesden who identified, quite unjustifiably, the non specific leprous sore with disease called in Latin morbilli. This term was a diminutive of morbus, meaning disease, which referred to the major disease. In the angilicized form of misellus, namely measels, the word hence forward became applied to the specific disease morbilli (Wilson G.S. 1962).

The disease was probably first recognized by Rhazes, a tenth century Arabian Physician and its identity as a specific disease was fully established by sydenham in the 17th century. Koplik in 1896, was able to establish a definite clinical basis for differentiating measles from rubella and other exanthems. Enders et al (1959), succeeded in cultivating measles virus and thus providing a relisble procedure for diagnosis and for prepration of vaccine. The vaccine was first used in clinical trial in U.S.A. in 1959.

MAGNITUDE OF THE PROBLEM

Measles is trully a universal disease, endemic throughout the world, sporadic cases occur throughout the year in all countries but epidemics are most freequent during the late winter and early spring. In large cities it is common for it to show a biennial peak, dependent presumably on the accumulation of susceptible persons. In Britain, Europe and the United States, measles has been a disease mainly of winter and spring. In India the peak incidence has been reported from March to June every year (Taneja, P.N. 1962).

EPIDEMIOLOGICAL OBSERVATIONS

Panum did classical studies on the epidemiology of measles in 1947. The attack rate in measles is higher than for any other infectious disease (Wilson, G.S. 1962). The disease is fairly mild in itself unless complicated by respiratory infectious when it becomes severe or even fatal. In virgin population that have not experienced a previous visitation, more than 90% of that population will be infected, Morley David (1975). Most children exposed to the infection for the first time contract the disease, with an infection to illness ratio almost one. Secondary attack rates over 90% was observed by Krugman and Ward in 1973, after intimate household exposure. However in the periodic epidemics of measles, in rural communities in India, attack rate in children born subsequent to the previous epidemics have been much lower (Pereira and Benjamin 1972, Siddique et al, 1974, Sinha, S. 1977, John T.J. et al., 1980). This may be due partly to limited exposure.

MORBIDITY AND MORTALITY PATTERN IN MEASLES

There have been reported a high morbidity and mortality in measles and a number of complications later on. Children with measles who do not recover may succumb to acute complications (mainly respiratory) or chronic disease (respiratory and neurological) may develop.

(Coovadia et al., 1977).

Despite the reduction in mortality morbidity is still high with 90-95% affected by the age of 10. Warin, J.F. (1967) reported a mortality of .2 per 10,000 notified cases in developed countries.

Measles in still a major cause of death in developing countries (Morley, D. 1969). In Africa mortality among hospital ranges from 5-25%. Hendrickse, R.C. (1975) has claimed a case fatality rate of 5% or more from developing countries. Koster, F.T. (1981) observed an case fatality rate of 3.7% in his study on measles in Bangladesh. In other developing countries the reported data are Guatemala (6.6%), Nigeria (5%), Tanjania (13.7%) while in developed countries such as U.S.A. (0.02%).

Studies from Asian countries and particularly from India, have shown, measles to be less severe as compared to [illegible] countries.from a rural community in India, Palghar Shah and Udani, P.M. (1969) have reported only 1.5% of the deaths due to measles, while Krishnamurthy, K.P., Dorai Rajan, R. (1979) observed a mortality rate of 11.76%. In a recent study Thomas cherian, Abraham Joseph and John T.J. (1984), while

carryingout their study in a measles epidemic in Tamilnadu (Dec.79-March,80), observed on attack rate of 54% and case fatality rate of 10%.

PASSIVE IMMUNITY IN MEASLES

Infants under 3 months of age are absolutely immune to measles and those between three and six or eight months are relatively immune. Infants acquire immunity transplacentally from mothers, who have had measles. Infants born to mothers immune to measles are protected against infection during their first 6 or 7 months after birth (Mehata, M.A. 1972). With the decline of maternal antibodies, babies become increasingly susceptible after 6 months of age and may on exposure develop disease of varying severity. Those with modified or occult illnesses are thought to be examples of partial protection by residual transplacentally acquired antibody. The infants of rare mothers who has never had measles or vaccine is susceptible at birth and may acquire the infection at any time postnatally.

AGE RELATION TO MEASLES

The incidence of disease is usually highest in the second, third and fourth years of life (Wilson G.S. 1962).

The peak incidence of the disease in developing countries is between 1 and 3 years. While in United Kingdom and Europe it is 5 years and above, and in United States it is 10-14 years (Morley D. 1969).

The extent of the prevelance of disease the immune status of the population, and the susceptible age at which infection generally occurs in India are unknown. In India median age of measles has been found to be different by different workers. Mehta, N.A. (1972) while conducting the serepidemiology of measles in Bombay found 48% positivity by 4 years of age and 100% by 7 years of age. Thus highest susceptiblity and attack rate was seen in the preschool and early school years.

Krishnamurthy and Ananthraman (1974) found highest incidence in between 1 and 3 years of age. Ramkrishnan et al (1978) observed maximum incidence in the age group of 0-1 years. Khan et al (1979) observed, onset of measles infection at preschool age (2-4 years) with the maximum rate of infection in school going age group 6-7 years. Bhaskaram P. et al (1984) found maximum incidence (45%) in children between 1 and 3 years, 22% in the age group less than one year, 20% in 3-5 years age group and rest above 5 years.

VIROLOGY

The pathologic agent causing measles is the paramyxovirus belonging to the group of myxeviruses. It's internal component of ribomucleic acid (RNA) with in a helliele protein capsid is enclosed by an outer membrane of a lipid and protein. It is about 140nm in diameter only one antigenic type is known. During the prodromal period and for a short period after the rash appears, it is found in nasopharyngeal secretions, blood and urine. It can remain active for at least 34 hours at room temperature.

The virus has been cultured in human and monkey leucocytes (Berg and Rosenthal, 1961).

Epidemiologically measles has been considered to be a respiratory disease since Panum (1938-39) and Babbot F.L. and Gorden J.E. (1954) first presented evidence for this point of view. It is assumed that infectious droplets of nasopharyngeal secretions from a patient land upon the respiratory epithelial cells of the new host. Infection occurs and the chain of events resulting in disease is initiated. It has been proposed particularly by Papp, K. (1956) that the primary site of infection is conjuectiva and evidence has been presented that the introduction of immune serum into this space or covering the eyes will protect against natural infection (Robbins, Fredrick, 1962).

PATHOGENESIS OF DISEASE

The classic investigations of Fenner, F. (1950) on the pathogenesis of measles in mouse pox, provide an experimental model with general applicability.

The sequence of events based on the Fenner scheme and making the proper adaptations for measles would be as follows.

Day 0	1-	Invasion of respiratory epithelial cells and multiplication.
Day1+	2-	Extension to regional lymph nodes.
Day2	3-	Primary viremia - This has not been conclusively demonstrated for measles.
Day3-5	4-	Multiplication in lymphoid tissues and respiratory epithelium with formation of giant cells; infection of respiratory tract probably mediated through the blood.
Day 5	5-	Secondary viremia.
Day7+	6-	Establishment of infection in skin, involvement of brain may result from virus reaching it, through the blood.
Day 11+	7-	Onset of prodromata.

Day 14+ 8- Development of rash.

Day 15+ 9- Antibody appears, viremia ceases and viral content in organs diminishes.

Day 17+ 10- Symptoms ameliorate, and rash begins to fade.

CLINICAL MENIFESTATIONS OF ILLNESS

Measles is characterized by three well recognised stages:

1- An incubation period of approximately 10-12 days with few, if any signs and symptoms.

2- A prodromal stage with an exanthem (Koplik spots) on the buccal and pharyngeal mucosa, mild to moderate fever, Slight conjuctivitis, Coryza and an increasingly severe cough, and

3- A final stage with a maculopapular rash erupting successfully over the neck and face, body arms and legs and accompnied by high fever.

MEASLES ANERGY, AND PROVOKING EFFECT OF THE DISEASE

The altered reactivity of the patient during measles is expressed in the state known as measles anergy.

Before the appearance of rash till the late convelescent, passive tuberculin reaction disappears,

(Von Pirquet 1908, Starr and Berkovitch 1964), the titre of immune bodies falls, the complement titre falls, the immunization capacity of the patient diminishes, and a negative schiek reaction changes to positive. As a result measles can light up latent infections (tuberculosis, dysentry, whooping cough etc.). The protective reaction of the child is lowered, the mild infections can become lethal. This provoking effect is particularly pronounced in children.

Tuberculosis and moniliasis infections normally controlled by cell mediated immune response are known to follow measles (Beck 1962, Smythe et al 1971).

MEASLES AND MALNUTRITION, ITS SYNERGISTIC ROLE AND COMPLICATIONS

Several clinical studies have high-lighted the synergistic effects of measles and malnutrition on the host (Gorden, J.E. 1965, Morley, D. 1969). Defence reactions are suppressed in malnutrition and mild infections can become lethal (Smythe P.M. et al., 1971). Children with measles who do not recover, may succumb to acute complications (mainly respiratory) or chronic disease (respiratory and neurological) may develop (Coovadia, H.M. et al., 1977). Prolonged

morbidity due to secondary infection is freequent especially in malnourished children and this has been attributed to immune-suppression.

MEASLES AND ITS COMPLICATIONS

Measles virus infections are associated with number of complications accredited to immune phenomenon glant cell pneumonia due to direct viral invasion of the pulmonary parenchyma is seen primarily in previously immunocompromised children (Maccarthy K. Mitus F., Cheathan W. et al., 1958). Secondary bacterial pneumonia and otitis media are freequent complications in otherwise normal children and are thought to related to virus induced immunosuppression (Miller, D.L. 1964), on the other hand the encephalitis seen in measles has been suggested to have an autoimmune basis (Koprowski, H.J. 1960, Lachmann, P.J. 1974).

Bhaskaram P. et al (1984) concluded that morbidity due to measles show two distinct phases. The children may develop complications in the acute stage of measles or during the subsequent period. The follow up study showed that the children suffered from freequent infections even after the attack of measles. This could be due to prolonged immune-suppression induced by the disease.

IMMUNOLOGIC SYSTEM

Immunologic system is the part of host defence, its primary function is to protect against invasion by infectious agent. The major cost of this protection are allergy, autoimmunity and rejection of organ transplant. There are four major limbs of immunologic system, T lymphocyte B lymphocyte, phagocyte and complement.

T AND B CELL LYMPHOCYTE

Harris et al (1945) had shown that the lymphocytes were involved in the immunological mechanism. It is now recognised that lymphocytes form an indispencible component of body immune status and embodies that precursor of cells that will give rise both cell mediated immunity and humoral immunity.

Study of Claman et al (1966), Devis et al (1967) Miller and Michel (1968) indicated that at least two population of lymphocytes were involved in most of immune response. These two population of lymphocytes are currently known as T cell (Thymus dependent), and B cell (Bursa equivalent derived), Rittest et al (1969). Graves et al (1973) had shown that T cell appear to be concern with cell mediated

immunity (CMI) and B cell with humoral immunity.

T lymphocytes play a major role in immune response to facultative organisms, tissue or organ graft and certain infections with viruses, B lymphocytes mature to become antibody producing plasma cells and play a role in humoral immunity response (Rowland (1975) lymphocytes circulate 4 to 6 times a day. T cells accounts for as many as 70% of peripheral blood lymphocytes while 20-25% are B cells (Lukes et al., 1974).

T lymphocytes are grouped broadly into modulater cells, effector cells, and cell producing lymphokines, modulator cells are further devided in to two catagories. Those that initints (helper or inducer) and those that tends to terminate (supressor cells) immune response (Reinherz and Schlossman 1980). The production of antibody by B lymphocytes requires the participation of helper T cells. A possible mechanism for subsequent termination of antibody production is the activity of suppressor cells. There appears to be a subpopulation of inducer T lymphocyte required to induce the function of the T suppressor lymphocyte (Morimote et al., 1981). In addition to modulatory lymphocyte there are the T lymphocyte called cytotoxic effector cells. These cells are able to recognise

foreign or altered self antigen, on the surface of cell and to destroy the cells (Paul W.E. 1980). The other function of T lymphocytes is secretion of lymphokines, these low molecular weight substance secreted by activated T lymphocytes, affect the function of other cells in the surrounding environment. T cells secretes one type of interferon, a lymphokine that stimulates other cells to develop anti viral activity. Macrophage migration inhibition factor secreted by stimulated T cells causes activation and immobilization of macrophages at the site of an inflammatory response (Rocklin at 1980). Inter leukin-2 is lymphokine that promotes activation and division of other T lymphocytes (Gillis 1983).

B lymphocytes has immunoglobulin molecules of a single antigenic specificity on its surface, when exposed to the relevant antigen usually processed by a macrophage, and under the influence of singnals from an antigen specific T lymphocyte, B lymphocytes differentiate into plasma cells which secretes antibody of same specificity as originally found in its progenitor. B cells are commonly identified by immuno globulin on SI-gm marker. Approximately 10% cell carry these markers along with IgD. The most commonly employed test

for B cell function is quantitative measurement of serum immunoglobin by single redial diffusion.

T AND B CELL COUNT

T and B lymphocytes can be indentified by various methods, antibodies against T and B cells have been prepared. But the most widely used method at present for identifying human T cell depends upon their ability to bind sheep RBC spontaneously in characterstic morphological configuration termed as rosette (Funden Berg 1975). Human B cell possess surface immunoglobulin detectable by direct immunoflorescence. They also possess receptor for aggregated immunoglobulins. For antigen-antibody complex and for the third component of complement. These receptor are detected by Erythrocyte coated with antibody or complement that surround B lymphocyte in cluster (Wybran and Fundenberg 1973).

Presently the spontaneous formation of rosette with sheep erythrocytes appears to be a specific property of T lymphocytes, and membrane bound immunoglobulin detectable by immunoflorescence constitute the most reliable marker of B cells, (Saligman 1974). However the fundamental nature of rosette formation is not known. They also possess receptor aggregated immunoglobins

for antigen and antibody complex and for the complement C_3, surrounded by B lymphocyte in cluster (Mendas et al 1973). These receptor are detected by erythrocyte coated with antibody and complement.

Steel, C.M. et al (1974) noted that T lymphocyte and B lymphocyte rosette formation are affected by temperature, incubation time, red cell to lymphocyte ratio, and sheep from which RBC are obtained. A short incubation time between the sheep RBC, and human lymphocyte result in rosette formation of only some of T cell where as a longer incubation time permits all T cell to bind. Thus the studies using longer incubation time usually have higher value of percentage of cell which form rosette. Fundenbergs and associates (1975) termed the population detected by short incubation period, active cells because they appears to be a subpopulation of more actively envolved in cellular immunity than total T cell population.

It is believed that rosette are formed by rapid release or metabolised receptor substance on the living cell surface. Positive bivalent ion are required since ethylene dismine tetra acetic acid will block to rosette formation (Jondal 1972), although comparable result using either ethylen dismine tetra acetic acid (EDTA)

or heparin a anticoagulant for rosette testing have been reported. Fair banks (1976) and Had field and associates (1975) reported that as the concentration of heparin was increased in the test system the percentage of T lymphocyte rosette decreased. Normaly there are more then 1500 circulating T cell/mm^3 each having less than 10 u in diameter. In some T cell deficiancy, number of lymphocyte count is normal or even elevated but the lymphocyte are larger than 10 u in diameter, monocytosis and eosinophilia are commonly associated with T cell deficiency (Nelson 12th edition).

VARIATION OF LYMPHOCYTES COUNT WITH AGE AND SEX

In study of deviation of T lymphocyte and B lymphocyte counts in disease, most report compared the data from so called normal population without specifying their normal characterstics though Elhilali and associate (1976) emphasized that importance of using age matched control in their study.

Zacharski and co-workers (1971) noted that there are no significant variation of lymphocyte count with sex or at various period of age. Wybran et al (1972) found that there is no difference in T and B cell percentage of infants and children. Wkesler and

Rutteroth (1974) found no difference in total lymphocyte and relative number of T lymphocyte in peripheral blood of young children and adult individuals.

NORMAL DISTRIBUTION OF T AND B LYMPHOCYTE

Neiburger et al in 1976 studied the distribution of T and B lymphocyte in peripheral blood of children and adult the found the following distribution:

	T Cell %	B Cell %
Children	44±4.2	30.4±3.1
Adult	46.3±1.8	26.5±2.3

Fleisher, T.A. et al (1975) studied the sub-population of lymphocyte in children and adult using E and EAC rosette assays. Children under 18 month of age were found to have less percentage of E binding T lymphocyte and an more percentage of EAC binding. B lymphocyte as compared to older children (18 month to 10 years) and adult. The absolute number of both E binding and EAC binding lymphocyte was more in children under 18 month of age than older children and adult, observation was as following

T(E Binding) Cell

Age groups	Percentage Mean±SD	Range	Absolute Number Mean±SD	Range
/18 month	50.2±8.7	33-67	2.970±690	16.20-4,330
18 month -10 years	56.9±5.9	45-69	1840±640	590-3090
Adult	64±69	51-78	1910±590	750-3070

B(EAC Binding) Cell

Age groups	Percentage Mean±SD	Range	Absolute Number Mean±SD	Range
/18 month	26.2±6.3	14-39	1530±540	470-2,590
18 month -10 years	22.7±3.4	16-29	720±280	170-1270
710 years	17.2±3.1	11-23	540-170	170-510

T AND B LYMPHOCYTES IN INFECTIONS

Niklasson et al (1974) found that patients with soute bacterial diseases and viral diseases have low percentage of T cells in their study of T and B lymphocytes in acute infections. The decrease of T cell in viral infections however was much more marked then bacterial infections. The active T lymphocyte values were usually decreased in viral illnesses but remain normal in bacterial illnesses.

B lymphocyte were found raised in both viral and bacterial illnesses, but the rise of B cell was earlier (Ist week) then bacterial illnesses (IInd week).

MEASLES AS AN INDEX OF IMMUNOLOGICAL FUNCTION

The measles virus has long been known to suppress immunological responses. Natural measles infection suppresses both cell mediated and humoral immune response (Whittle, H.C. and Bradley Moore et al., 1973), and this coupled with malnutrition, leads to the death of many children from secondary infection (Morley, D. 1969). In 1908 Von Pirquet reported that the tuberculin reaction was suppressed in children with measles who had previously been positive to this test. Subsequent studies have also shown that extensive immunosuppression exists during acute measles (Coovadia et al., 1978).

White, R.G. and Boyd, J.F. (1973) attributed this immunosuppression to the wide spread aggregative destruction of thymocytes seen in the cortex and medulla on the fourth day of disease. Tuberculin hypersensitivity can disappear before the date of onset of measles rash and be absent after measles vaccination for an average of 18 days (Starr and Berkovitch, 1964). According to Smithwick and Berkovitch (1966), transformation of lymphocytes from Montoux-positive subjects by tuberculin PPD was depressed by addition of measles virus to the tissue culture. However, PHA could effect transformation

in a normal fashion of the same virus treated cells. (Osunkoya et al., 1974). Finkel and Dent (1973) noted impairement of lymphocyte response to sub-optimal dose of PHA.

Other hypothesis for depression of cell mediated immune response has been advanced.

Osunkoya et al., (1974) have revealed the presence of measles virus in lymphocyte during acute infection judged by immunoflorescence. They also suggested that depression of CMI response could be cause of a transient reduction in number of T lymphocyte as a result of cytopathic destruction.

Joseph et al., (1975) have shown that in vitro both T and B cells and monocytes can be infected by measles virus. Kantor, F.S. (1975) raised the possiblity that the virus might stimulate some lymphocyte to release a suppressor of cell mediated immunity.

Whittle, H.C. and Dossetor, J. (1978) were able to revover virus directly from lymphocytes which support the impairement of CMI response. Palton, B.K., Hylton Winsome et al., (1982) have shown that a small percentage of both T and B lymphocytes are infected, but like HSV, measles virus only suppress the inductive stage of a

specific antibody or immunoglobin response.

Controversies still exist regarding the mechanism of immunosuppression, especially in young children. A decrease in helper/inducer (H) to suppressor/cytotoxic (S) T-cell subpopulation ratio (H/S ratio) resulting from a decrease in H counts was found in adults (Alpert et al., 1985) and in one study in well nourished African children (Jeffe et al., 1983) during acute measles infection. In contrast, other did not find any change in H/S ratio during acute measles in older children and young adults despite a decrease in both H and S counts (Arheborn and Biberfeld 1983).

It is now well established that cell mediated immunity is important not only for recovery from measles but also for resistance other bacterial and viral infection (Bhaskaram and Reddy, V., 1983). They have shown that cell mediated immune response was observed to be significantly depressed in children following measles.

It is concluded from above studies that measles process can itself demolish a preexisting state of cell mediated immunity. The resulting deficiency of thymus dependent lymphocyte, as a result of replication of virus with in or cytopathic destruction or loss of discernible

cortex from the thymus (White, R.G. and Boyd, J.F., 1973), may be sufficient to impair the specific immunological attack on the virus and allow persistance of large amount of virus in the thymus (Burnet 1968). Indeed the extensive destruction of thymocytes in the thymus gland may be the major factor in development of a state of tolerance to measles-antigens predominantly in respect of cell mediated immunity as was postulated by Burnet (1968).

It has also been reported that a preexisting state of malnutrition might produce diminution of cell mediated immunity via reduction in the population of thymus- dependent lymphocytes (Smythe, et al., 1971), which could predispose to severe or fatal measles.

The whole process of the eruptive stage of measles and subsequent immunity is mediated by the thymus dependent system. Burnet, F.M. (1968) hypothesized that in the course of the generalized delayed hypersensitivity reaction which we see as the measles rash, there is discharge and exhaustion of all those local cells probably including mast cells, which can contribute pharmacologically to the local reaction. He further added that it could also be assumed that the

regions from which large number of T-D (Thymus-dependent) cells are liberated, have been temporarily exhausted. Taken together these are responsible for the failure of the classical Montoux reaction to be elicited in the weeks following measles. He stated that this phenomenon is a clear indication of the fact that the measles rash is itself a diffuse delayed hypersensitivity reaction. The whole pattern of measles pathogenesis and immunity is clearly based on thymus dependent immunocytes. Measles is infact a complex and severe delayed hypersensitivity reaction. The Gut dependent (GD) system and its antibody are side effects epiphenomenon of minimal or no importance.

T AND B CELL STUDIES IN MEASLES

Various studies have shown the effect of measles on number of circulating T and B lymphocytes, null cells. Complement C_3 and antibodies and immunoglobins. Lymphocyte studies have been done using different surface markers on T and B lymphocytes namely E and EAC rossette technique, monoclonal antibodies OKT-3 and Quantigen beads etc.

Coovadia et al (1977) have found that the profound immuno-suppression during the first few days of the rash in measles which can determine prognosis has

been shown to affect chiefly the T and B cell subpopulations with less severe effects on C_3 and T cell function assessed by PHA transformation of lymphocytes. They have carried out the immunological studies in two groups of measles patients. Group A in which there was severe lymphopenia ($\angle 2000/mm^3$) and Group B in which lymphocyte count was ($\geq 2000/mm^3$). Subpopulations of lymphocytes were counted in a single prepration by means of sheep rossette formation and by an immune-floroscence method for detecting immunoglobins. Peripheral lymphocytes were classified as rosetting cells (T) florescing cells (B), cells with no markers (null) and those with both markers (FT). The distinguishing pattern in absolute lymphocyte counts, T.B. null and FT cells was observed. The mean initial absolute lymphocyte counts in Group A ($1318 \pm SEM89/mm^3$) and Group B ($4232 \pm 314/mm^3$) were lower than that in healthy control ($6833 \pm 553/mm^3$).

Lymphocyte subpopulation except for null cells had reached the levels of normal controls at the third week after onset of rash in those who recovered. At the third week of the rash lymphocyte subpopulation except for F.T. cells, were still significantly below normal in children who did not recover. At the sixth week only the T cells in addition had reached normal in these children

where as the absolute lymphocyte counts, B cell and null cells were still significantly depressed.

Whittle, H.C. and Dossetor et al. (1978) have shown in their study of 25 children with natural measles that the number and proportion of circulating T lymphoctyes was low in the acute stage of measles. 37% of T cell showed positive immuneflorescent staining for measles virus after stimulation with PHA. 7% of the B cells were shown to contain virus, but their number did not alter significantly during the infection.

Group	T cells	B cells	Null cells
Acute measles	38.7±13.8	32.7±8.4	26.7±16.5
4 weeks later	42.2±7.1	29.9±6.0	27.9±9.5
Controls	53.3±10	32.3±9	14.4±10.3

Pelton, Winsome Hylton (1982) have shown that both T and B cells are infected in disease process and both cell types support measles virus replication.

Joffe, Max I and Sukha Nagin, R. et al. (1983) observed a depression in circulating T lymphocytes using monoclonal OKT 3 antibody and Quantigenbeads, as well as enumerating E-rossette forming cells.

Measles patient	Lymphocytes	E rosette %	OKT 3% Monoclonal-antibodies
1	4400	41	38
2	1240	52	48
3	4030	44	44
4	1350	50	43
5	1100	32	34
Controls			
1	2400	70	66
2	3750	57	62
3	2850	65	62
4	3800	68	65
5	2950	60	68

Bhaskaran, P. et al. (1983) studied 34 children aged between 6 months and four years. Cell mediated immune response was observed to be significantly depressed in children following measles. The degree of immunosuppression was found to be significantly depressed (31.1±1.55% T cells) in well nourished children and 32.4±2.21 in under nourished children.

Nutritional status	No. of children studied	T cell %		
		Initial count	3 months	6 months
Normal controls	22	% Rossette 49.0±1.94		
Well nourished children with measles	20	31.1±1.55	31.3±0.97	42.2±0.98
Under nourished children with measles	11	32.4±2.21	32.8±1.21	43.3±1.76

Arneborn and Biberfeld (1983) observed a depression in total T subsets (leu 4) during the acute phase of measles as compared to normal controls.

Monoclonal antibodies

Leu 2 a - suppressor/cytotoxic

Leu 3 a - Helper subset of T lymphocytes

Leu 4 identifies Total T cells.

Measles Pt.	Leu 2a	Leu 3a	Leu 4(Total T cells)
1	15	28	44
2	14	49	69
3	21	42	58
4	25	42	64
5	34	40	68
6	23	32	51
7	28	41	61
8	17	45	63
Controls	25	45	79
Median	16-38	38-59	66-83

Per Arneborn and Gunnel Biberfeld (1983) have found that in acute phase of measles, there was T lymphocytopenia but no change of the ratio between T lymphocytes of helper and suppressor/cytotoxic cell phenotypes.

Robert, L. Hirsch et al (1984) have shown that lymphocytes from patient with measles showed profound and prolonged suppression of proliferative response to mitogens. The degree of suppression was similar in patients

with uncomplicated measles virus infection and in those with pneumonia or post infectious encephalitis.

IMMUNE RESPONSE IN MALNOURISHED Vs WELL NOURISHED CHILDREN

In malnourished children measles is often severe and can be fatal in upto 50% of cases (Whittle et al. 1980, Dossetor et al., 1977). Many speculate that this phenomenon is due (Anonymous 1982, 1983) at least in part, to the impairement in cellular immunity observed in malnourished children especially during measles. However Whittle has demonstrated that in malnourished children although peripheral blood mononuclear cells (PEM) support a higher replication of measles virus, their cellular immunity does not seem to differ firm that in well nourished children (Dossetor et al., 1977). It has been suggested (Whittle et al., 1980, Dossetor et al., 1977) that lymphocytes of children with malnutrition are abnormally susceptible to infection by measles virus. The infection is followed by a normal cellular and humoral immuneresponse and this response generates immunosuppressive factors in the patients plasma, thus making the child susceptible to secondary infection (Ron Dagon et al., 1987, Bhaskaran, P. and Reddy, V., 1986) investigated the effect of PEM on the clinical course, outcome and immune status of 50 children with different

nutritional status. The duration and complication of measles were found to be similar in well nourished and malnourished children.

Nutritional status % of standard weight Weight/age	No.	% of T cells
⩾90%	Measles (8)	31.2±0.84
	Control (12)	62.5±1.87
76-90	Measles (15)	29.8±0.98
	Control (15)	60.8±1.94
⩽60%	Measles (12)	25.1±1.76
	Control (15)	28.6±1.22

The immunological parameter showed that the percentage of circulating T lymphocytes was significantly lower in severely malnourished compared to those of other nutritional grades. However, children with measles irrespective of nutritional status showed a significant decrease in the circulating T cell number compared to the controls. Severely malnourished children with measles did not show any further decrease in the T cell number compared to their matched controls.

Ron Dagon et al (1987) investigated the effect of measles in malnourished and well nourished children, and observed that malnourished infants showed a trend towards a deeper depression in both helper and suppresser T cells during the acute phase than well nourished children where as the helper/suppressor ratio remained similar in two groups.

There was a more impressive decrease in mean T lymphocyte counts than in B lymphocyte count in children with measles.

Variable	Patients acute phase	Patients Convalescent phase	Controls
	N = 28	N = 19	N = 22
Total WBC	8.581±3.290	9.795±2500	
Total lymphocytes	3.444±2065	4.765±1545	
B lymphocyte Mean %±S.D.	16±6	21±8	14±3
T lymphocyte Mean %±S.D.	54±10	60±14	64±14

REVIEW OF T CELL FUNCTION

Human T lymphocytes are endowed with the capacity to recognize specific antigens, execute effector functions and regulate the type and intensity of virtually all

cellular and humoral immune responses (Reinherz, E.L., Schlossman, S.F. 1980).

Two major functionally distinct subsets of T cells have been defined with heteroantisera, autoantibodies and monoclonal antibodies directed at stable cell surface antigens (Evans, R.L. et al., 1978, Reinherz, E.L. et al., 1980).

The human immune system, therefore consists of discrete subsets of T cells that are critical for immune homeostasis. It is the balance between effector and regulatory subsets that governs the outcome of antigen triggering. The inducer subset is central for the activation of T and B cells, and macrophages, as well as for haematopoietic differentiation. This inductive influence is regulated by the presence of suppressor T cells that function to inactivate the inducer subset or alternatively, the effector itself population. Loss of activation of these subsets leads to a variety of immunologic disorders characterized by autoimmunity or immunodeficiency. Immune homeostasis results from a delicate balance of inducer and suppressor subsets with in the human T cell circuit.

ASSESSMENT OF T CELL FUNCTION

A popular method to assess the function of T cells in vitro is to quantitate the amount of cell division (Blastogenesis). They undergo the process in response to stimulation in vivo by specific antigen or by mitogen (Plant derived material) that purturb the lymphocyte membrane and triggers the cell division. In vivo T lymphocyte function can be measlured by delayed hypersensitivity reaction using variety of antigen to which majority of older children and adult have been sensitized. The most generally useful skin test antigens are 1:100 dilution of tetanus toxoid, PPD, histoplasmin mumps, extract of candida, trichophyton, PHA and DNCB.

Skin tests are more important test for CMI assessment in vivo, appropriate antigen skin test were evaluated by different observers to assess erythma, oedema in course of time as well as size of reaction, they can provide the valuable information. Positive skin test are of value in establishing the presence of normal T cell function but negative skin test are inconclusive evidence of deficient T cell function.

ALTERED CUTANEOUS HYPERSENSITIVITY REACTIONS FOLLOWING MEASLES

Many workers have studied the effect of different mitogens PHA, PPD, streptococcal antigen, PWM etc. on lymphocyte transformation in measles.

Sellamayer Erica, Bhettay E. et al (1972) studied 7 patient of measles and observed, lymphocyte transformation to be more depressed in measles patient than in other diseases. The responses in the measles group were uniformly lac and significantly less than in the controls (P/0.001).

Whittle, H.C. and Bradley Moore et al (1973) have demonstrated that delayed hypersensitivity to specific antigens like PPD and candida and streptococcal antigens is temporarily suppressed in measles. However when the expression of delayed hypersensitivity was suppressed the patient could still be sensitized normally to DNCB and PHA. 31 of the 33 patient (94%) previously sensitized with 2mg DNCB responded to a challange of 200 ug DNCB and their lymphocytes responded to stimulation with PHA.

Group	PPD		Candida		Streptococcal antigen	
	Day		Day		Day	
	3	16	3	16	3	16
Measles(No.33)	0	6 P<0.05	6	19 P<0.01	3	14 P<0.01
Control(No.34)	4	ND	21 P<0.01	ND	13 P<0.02	ND

On day 3rd after measles rash no patient responded to PPD. 6 patient responded to candida and only 3 patient to streptococcal antigen. After repeat skin testing; read on day 16 the number of reactions in the measles group was comparable to controls.

Bhaskaran, P. et al (1983) investigated the effect of measles infection on the nonspecific response to mitogens and observed a significant reduction of PHA induced lymphocyte response as judged by DNA synthesis.

Arnebern - Per and Gunnel Biberfeld (1983) found a low proliferative response to PHA during the acute phase of measles and varicella. The response to PPD was also low in all measles patient tested and in some of the varicella patient.

Hirsch Robert, L. et al (1984) in their study observed that lymphocyte from patients with measles showed

profound and prolonged suppression of proliferative responses to mitogens (PHA, PWM and PBS). The degree of suppression was similar in patients with uncomplicated measles virus infection and in those with pneumonia or post infectious encephalitis.

Madhusudan and Bhaskaran, P. (1986) studied the response of T cells to PHA and to measles antigen and found a low CMI response to PHA in children with measles irrespective of their nutritional status indicating the effect of measles perse on immune status.

Ron Dagon, Moshe Philip et al (1987) observed a reduced response to mitogens (PHA, ConA and PWM) during the acute phase of measles mean % of stimulation (±SE) by PHA ConA and PWM were 81±8, 71±11 and 58±11 respectively during the acute period most of the workers have shown matogen stimulation response to lymphocyte, to be reduced. However Whittle, H.C. et al have shown PHA stimulation to be normal.

As a test for cellular immunity, contact sensitization to 1-nitro, 2,4-dichlorobenzene (DNCB) offers several advantages over intradermal tests. Reliance upon previous exposure to the allergan is unnecessary since both sensitization and challenge are controlled and approximately 95% of normal people can be sensitized to

this agent, (Krugman, A.M.l Spatein, W.L. 1959). Furthermore circulating antibodies do not develop with contact sensitization (Waksman, B.H., 1960) which renders it a more exact test of cellular immunity.

The sensitizing properties of DNCB are related to its abslity to act a hapten forming convalent bands with lysine groups of epidermal protein (Eisen 1958). A threshold concentration of DNCB in required for sensitization. Less then 5% of the applied DNCB becomes bound and a relatively brief duration of binding in the skin is necessary. Sensitization takes place in the regional lymph nodes and is mediated by circulating lymphocytes. The development of sensitization requires seven to twenty one days.

The capacity to become sensitized to DNCB may be tested by application of DNCB to the skin, followed 2 weeks later by patch testing at different sites. Thus ability of an individual to develop CMI denove can be determined by applying DNCB directly to the skin. The chemical combines with skin proteins to forma immunogenic substance that stimulate sensitization of T cells to DNCB, 10-14 following this initial exposure to DNCB the reapplication of DNCB on skin will result positive skin test if CMI is intact.

Sanjeev, Rai P. Krishnamurthy, P.N. et al (1981) carried out DNCB skin sensitization test in 170 malnourished children and compared it with control group, their studies have shown DNCB reaction, positive only in 54.1% of malnourished children compared to 86.7% in control group.

DNCB Reaction	Control group			Study group		
	No.	%		No.	%	
3+	11	36.7		31	18.2	
2+	12	40	86.7%	49	28.9	54.1%
1+	3	10		12	7.0	
Negative	4	13.3		78	45.9	
	30	100		170	100	

Simultaneously they have also studied the pattern of reaction in various groups of malnourished children. The reaction was related to the degree malnutrition severe the malnutrition, more the negative reaction.

For DNCB skin test 1000ug/0.1 ml concentration were used for sensitizing dose and 50 ug/10.1 ml for challenge dose. Reaction was graded as under (Sanjeev Rai, P. and Krishnamurthy, P.N. et al., 1981).

3+ Contaneous flare occuring at both sensitizing dose and challenge dose sites.

2+ Spontaneous flare occuring at sensitizing dose site.

1+ Absence of spontaneous flare, but on reapplication of challenge dose an equivocal delayed hypersensitivity reaction.

Negative- No reaction, no spontaneous flare occuring even after reapplication of challange dose.

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MATERIAL AND METHOD

MATERIAL AND METHOD

The present study was conducted in department of paediatrics, in collaboration with department of Pathology.

Forty eight children with measles aged 8 months to 12 years were selected from the Out Patient Department of paediatrics and from those admitted in children ward of this hospital. The nutritional status of all measles patient was assessed according to Harward weight/age standard. Those having weight above 80% of the 50th percentile of Harward, were considered as well nourished and those who were below this reference median were taken as malnourished. The malnourished children were further divided into grade I,II,III and grade IV malnutrition group according to classification of Indian Academy of Pediatrics. The study children having 71-80% of expected weight were taken as grade I malnutrition children, similarly those having weight 61-70%, 51-60% and /50% of 50th percentile of Harward standard were classified as grade II, III and IV malnutrition children. The study group children were matched according to age with twenty normal healthy children. They were selected from the well baby clinic

and from the paediatric Out Patient Department. These children served as controls. With parental consent, 10 ml venous blood was obtained under strict aseptic precautions from measles patient during the first 0-6 days of appearance of rash, for immunological study. Similarly control group children were also investigated. Besides Name, age, sex, address and socioeconomic status of children, the present and past history of illness and family history were questioned in each case.

From parents or other family members detailed history was obtained regarding present illness in chronological order. History of associated complications like diarrhoea, dysentry, Whooping cough was asked. Bronchopneumonia and encephalopathy were diagnosed clinically. History of immunization viz. Polio, DPT, BCG and measles was interrogated. In the past history, history of worm infestations, asthma, pertussis and tuberculosis was interrogated.

In family history, history suggestive of chronic illnesses like tuberculosis and asthma was questioned in parents, Siblings and neighbourhood.

Physical Examination

Thorough clinical general examination and the examination of respiratory, Cardiovascular, gastrointestinal and central nervous systems was done in each case.

Weight

Weight was recorded nearest to 0.01 kg by using infant weighing scale for infants ⩽10 kg and adult type weighing machine for children ⩾10 kg.

MATERIAL REQUIRED FOR T & B CELL ESTIMATION

1. Heparin Preservative free.
2. Minimum Essential Medium (MEM) (Eagles).
3. Alsever's Solution

Glucose	24.6 gm.
Trisodium Citrate dehydrate	9.6 gm.
NaCl	50.04gm.
Distilled water	1200 ml.

PH was adjusted to 6.1 with 10% citric acid, sterlized by low pressure autoclaving and stored in refrigerator.

4. Phosphate Buffer Saline (PBS)

Phosphate Buffer Solution

(A) 0.15M - $NaH_2Po_4.2H_2O$) 23.4 gm/litre

(B) 0.15M - Na_2HPo_4 21.3 gm/litre

Normal Saline

NaCl 9.0 gm/litre

Phosphate Buffer Saline

For PH 7.4 - Solution A - 18 ml

and Solution B - 82 ml

and then normal Saline 100 ml was added, solution was sterlized by low pressure autoclaving and stored in refrigerator.

5. Pooled Normal Human Serum:- 15 ml Venous blood were drawn aseptically into clean and dry test tube from 4 persons. Then test tube incubated in water bath at 37°C for 30 mins. and then at 40°C for 120 mins. The clot removed gently with glass rod and test tube was centrifuged. The clear serum from each tube was collected and pooled together then stored at -20°C (freezing) in small aliquotes and used once after thawing.

6. Antisheep Haemolysin (Ambo Cepter) (SPAN Diagnostic).

7. Methylene Blue 0.2%

LABORATORY PROCEDURE:-

Collection of Sample:- Ten ml. heparinised peripheral blood sample (25 unit of Heparin/ml of blood) was collected in the sterile tube from each patient for B and T cell studies. Blood was simultaneously collected from these

patient for total and differential luekocyte counts in double oxalate vials.

Total Leukocyte Count (TLC):- One in 20 dilution of blood was made by adding 0.02 ml of blood to 0.38 ml of WBC diluting fluid (Turk's fluid) in 7.5x10 mm test tube. The suspension was mixed by gentle tilting and rotating by hand for 2 minuts. The Neubour's counting chamber was filled using pauster pipett. The preparation was viewed with 5 mm objective under microscope. The number of lukocytes were counted and calculated as below:-

TLC = N x 200/cu mm.

N is number of leukocytes counted in each mm square area.

Differential Leukocyte Count (DLC):- A thin and uniformly prepared peripheral blood smear was stained for 8 to 10 minuts with leishman stain, washed with buffered water and dried in air. Leukocytes were counted using oil emersion lens and the percent distribution of different leukocyte was calculated based on the count of 200 cells.

Absolute Lymphocyte Count (ALC):- Absolute lymphocyte count was calculated in every case from the total and differential leukocyte count using following formula.

$$ALC = \frac{TLC \times \% \text{ lymphocytes}}{100}$$

Estimation of Haemoglobin:- Estimation of haemoglobin was done by Sahli's Method.

Estimation of ESR:- Estimation of erythrocyte sedimentation rate was done by the Wintrope method, a haematocrit tube was filled to the 100 mm mark with oxalated blood and allowed to stand vertically for one hour.

EVALUATION OF T AND B LYMPHOCYTES

Preparation of Lymphocyte rich plasma:- The lymphocytes were separated from the heparinised peripheral blood by gravity sedimentation method. Ten ml of heparinised blood (25 unit/ml blood) collected in a sterilised test tube was kept up right at room temperature for one hour. The leukocytes rich plasma was collected and centrifuged at 1000 rpm for 15 minutes, the clear plasma was seperated and the cell button was suspended in minimum essential medium (MEM). The concentration of lymphocytes was adjusted to 2-3 x 10^6 per ml in MEM.

Preparation of Sheep RBC solution:- Sheep blood collected in equal volume of Alsever's solution was stored in refrigerator for 3 to 5 days and there after used upto 14 days. Sheep erythrocytes was washed thrice with buffer saline. One volume of packed cell was suspended in 19 volume

of buffer saline to give a slightly greater concentration than 5% suspension. One ml of this suspension was lysed with exactly 14 ml of distilled water and optical density (OD) was measured at 540 um with distilled water as blank. A lysate with an O.D. of 0.7 represented 5% or 1×10^9 cell/ml. From the O.D. of sample tested and volume of the suspension (Vi), the final volume (Vf) was calculated according to the relationship:-

$$Vf = \frac{Vi \times O.D.}{0.7}$$

Finally suspension was adjusted to make standard solution of sheep RBC.

Titration of haemolysin:-

This was first performed so that complement titration was independent of the concentration of haemolysin. 5.0 ml volumes of 5% SRBC are treated with equal volumes of 1 : 50, 1 : 100, 1 : 200, 1 : 400 and 1 : 800 diluted haemolysin in GVBS for 15 minutes at 37°C. The sensitized SRBC is now called EA.

6.5 ml volumes of 1 : 50, 1 : 100, 1 : 200 and 1 : 400 diluted normal human serum (NHS) are also prepared and then tubes are set up as shown in Table 1.

Row	A	B	C	D	E	F
	NORMAL HUMAN SERUM 6.5ml				OVES	DISTILLED
EA 1.0 ml	1:50	1:100	1:200	1:400	6.5ml	WATER 6.5ml
EA 1:50						
EA 1:100						
EA 1:200						
EA 1:400						
EA 1:800						

After incubation at 37°C for 60 min. the tubes were centrifuged and the O.D. measured at 541 nm. Pecentage of lysis was calculated by the formula :

$$\frac{\text{OD ROW A to D-O.D. ROW E}}{\text{O.D. ROW F}} \times 100$$

The dilution of antiserum which gave maximum haemolysis with 1 in 100 or 200 diluted human serum was used in subsequent titrations. This was carried out on all new bottles/batches of haemolysin.

Demonstration of T cell by sheep RBC Rosette (E Rosette):-

Sheep RBC were washed thrice with MEM and 0.5% suspension was made in phosphate buffer saline. Lymphocyte count was adjusted to 2-3 x 10^6 per ml in MEM. To 0.5 ml of sheep RBC suspension in test tube, 0.5 ml of lymphocyte suspension was added and mixture was incubated for 15 minute at 30°C in water bath. After centrifugation for 5 minute at 500 rpm, mixture was incubated at 4°C for over night. Supernatent was removed and pallet was resuspended in remaining fluid (2-3 drops). Finally wet preparation was made and stained with methylene blue. Rosette forming

lymphocytes out of 200 cells were counted under microscope and value expressed as percentage of rosette forming cells.

Three or more SRBC adhering to a lymphocyte were taken as rosette forming cells. The absolute T cell count was calculated as follows:-

$$\text{Absolute T cell count} = \frac{\text{ALC} \times \% \text{ T cells}}{100}$$

Demonstration of B cell by formation of EAC rosette:-

(Sheep RBC coated with anti sheep haemolysin antibody and complement).

To 0.5 ml of 5% SRBC suspension, 0.5 ml of anti sheep haemolysin in appropriate dilution (1:400 assessed earlier) was added and incubated for 15 min. at 37°C. After washing three times with phosphate buffer saline and resuspending in phosphate buffer saline and there after adding 0.5 ml of 1:10 diluted complement (human serum), tubes were incubated for 45 minute at 37°C. These cells were washed thrice with phosphate buffer saline and than resuspended to make a concentration of 0.5% EAC in phosphate buffer saline.

To 0.5 ml suspension of lymphocytes ($2-3 \times 10^6$ ml), 0.5 ml of EAC suspension in PBS was added and incubated at 37°C for 30 minute, the solution was resuspended and wet

preparation was prepared and stained with 0.2% methylene blue and rosette forming lymphocytes out of 200 cells were counted.

Three or more SRBC adhered to a lymphocyte were considered to be rosette. Absolute B cell count was calculated as follows:-

$$\text{Absolute B cell count} = \frac{\text{ALC} \times \% \text{ B cells}}{100}$$

2,4 Dinitro Chloro Benzene (DNCB) contact skin sensitization test:-

Stock solution of DNCB in acetone of 1000 ug/0.1 ml and 50 ug/0.1 ml concentrations was made and stored in amber coloured bottles at room temperature, this solution was changed after every three months. Stainless steel ring of 2 cms diameter was placed at the site of application of DNCB so that fixed area was obtained. Sensitizing dose of 1000 ug/0.1 ml was applied on the right upper arm on volar surface slightly towards medial side, simultaneously challange dose 50 ug/0.1 ml was applied on right forearm on flexor surface on medial side.

After the application of DNCB, these sites were covered and subjects were instructed not to wash the sites for 24 hrs., sites were examined after 48 hrs.

for irritative reaction and at 14th and 21st days for a spontaneous flare, indicated by appearence of erythema induration and vesiculations.

Reaction was graded according to criteria proposed by Sunjeev Rao, P. et al (1981) which are as follows:-

+++ Spontaneous flare occuring at both sensitizing dose and challange dose sites.

++ Spontaneous flare only at sensitizing dose site.

+ Absence of spontaneous flare but reapplication of challange dose eliciting an equivocal delayed hyper-sensitivity reaction.

-ve No reaction, No spontaneous flare occuring even after reapplication of challange dose.

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OBSERVATIONS

OBSERVATIONS

CELL MEDIATED IMMUNITY IN MEASLES

The present study was conducted in the Department of Paediatrics, in collaboration with Department of Pathology, M.L.B. Medical College, Jhansi (U.P.), between September 1988 to June 1989, to assess immunological status of children following acute measles.

Study was carried out on 48 measles children aged between 8 months to 12 years which included 15 well nourished and 33 malnourished children as assessed by Harvard weight/age standard. Twenty age matched normal healthy children belonging to different age groups were similarly investigated for immunological profile. Cell mediated immunity was assessed by E. rossette (T cell) count and dinitrochloro benzene skin sensitization test in each child of study and control group. Humoral immunity was assessed by EAC rossette (B cell) count.

Table I
Distribution of children

Group	No. of cases	Age range	Mean age	Mean weight
Control	20	8 month to 12 years	3.8±2.58	14.65±1.84
Study	48	8 month to 12 years	3.64±2.68	10.92±4.50

Table I shows the distribution of cases in control and study groups. Control group included 20 healthy children aged between 8 months to 12 years having mean age 3.8±2.58 years and mean weight 14.65± 1.84 kg. while study group comprised of 48 children aged 8 months to 12 years, having mean age 3.64±2.68 years and mean weight 10.92±4.90 kgs.

Table II

Distribution of control and study group children according to age.

Age group (Year)	No. of cases		Mean age	
	Control group (20)	Study group (48)	Control group	Study group
∠ 1	3(15%)	4(8.33%)	.823±14	.8±11
1-2	4(20%)	12(25%)	1.68±.26	1.58±20
2-4	7(35%)	20(41.6%)	3.21±.55	3.15±.35
4-12	6(30%)	12(25%)	7.08±2.68	7.45±2.70

Table II illustrates the distribution of control and study group children according to their mean age. The children of two groups were sub divided into four age groups viz. below 1 year, 1 year to 2 year, 2 year to 4 years and 4 years to 12 years respectively. The number of cases in control group were three, four, seven and six respectively, while study group comprised of four, twelve, twenty and

twelve children respectively of different age groups. The highest age incidence of measles depicted in this study was between 2 to 4 years i.e. 41-66 of the total study group children. The mean age of control group children was .823±.14 year, 1.68±.26 year, 3.21±.55 year and 7.08±2.63 year respectively. The mean age of study group children was .8±.11 year, 1.58±.20 year, 3.15±.35 year and 7.45±2.70 year respectively.

Table III

Distribution of Control group children according to age and sex.

Sex	No.of cases	%	Age Mean±SD	Weight Mean±SD
Male	13	65	3.80±3.01	15±6.64
Female	7	35	3.78±1.45	12.28±2.79

Table III depicts the sex distribution of control group children. Male children were 13 (65%) having mean age 3.80±3.01 years and mean weight 15±6.04. While female children were 7 (35%) having mean age 3.78±1.45 year and mean weight 12.28±2.79 kg.

Table IV
Nutritional status of measles cases

Nutritional status	No.of cases	%	Mean age (years)	Mean weight (kg.)
A: Well nourished	15	31.25	3.64±2.66	12.60±4.10
B: Malnourished				
Grade I Malnutrition	18	37.5	3.48±2.37	10.42±4.14
Grade II Malnutrition	10	20.83	4.17±4.08	10.19±5.52
Grade III Malnutrition	5	10.41	3.2±.20	7.4±1.81

Table IV shows the nutritional status of study group children viz. well nourished and malnourished as assessed by Harward weight/age standard. Children having weight 80% or more than 80% of the 50th percentile of Harward standard were classified as well nourished and those having weight less than 80% of the 50th percentile of Harward standard were termed as malnourished. They were subdivided into grade I,II,III and IV malnutrition based on classification of Indian Academy of Pediatrics children having weight 71-80% of 50th percentile of Harward were taken as grade I malnutrition children, similarly the children having weight 61-70%, 51-60% and /50% of 50th percentile of Harward were classified into

grade II,III and IV malnutrition group.

The number of well nourished measles children was 15(31.25%) while malnourished children were 33, of which 18 were of grade I malnutrition, 10 of grade II malnutrition and 5 of grade III malnutritional group respectively, no patient of grade IV malnutrition was available this study. The mean age of well nourished children was 3.64±2.66 year and the mean weight was 12.60±4.10 kg. The malnourished children of grade I, II and III group had their mean ages 3.48±2.37 year. 4.17±4.08 year and 3.2±1.26 year respectively. Their mean weight were 10.42±4.14 kg, 10.19±5.52 kg. and 7.4±1.81 kg. respectively.

Table V
Incidence of Complications

Type of complications	No.of cases	%
Respiratory	18	69.23
Gastrointestinal	8	23.07
Neurological	4	7.69
Total number of complicated cases	30	100.00

Table V reveals the type of measles associated complications for which patients sought the admission of the 48 measles children 30 of the cases (54%) were hospitalized with complications. Out of these 18 children (69.23%) had respiratory complications viz. bronchopneumonia, emphysema, laryngotracheobronchitis etc., 8 children had gastrointestinal complications mainly diarrhoea and dysentry, 4 cases had encephalitis.

Table VI
Incidence of complications according to age

Age range (year)	No.of patient	No.of complicated cases	%
< 1	4	3	75
1-2	12	8	66
2-4	20	13	65
4-12	12	6	50

Table V and Figure 1 depicts the number of complicated cases according to age. As illustrated above the infants were mostly inflicted with complications i.e. (75%), followed by children of 1-2 year age group (66%) than preschool children (2 to 4 year) (65%), and finally the school going age group.

Table VII

Morbidity pattern in well nourished and malnourished study group children

Nutritional status	No.of cases	Measles associated complications (No.of cases)	Measles cases without complications
Group A Wellnourished	15(31.25%)	5(33.3%)	10(66.66%)
Group B Malnourished	13(68.75%)	25(75.75%)	8(24.25%)
Total number of Measles patient	48	30	18

As illustrated in table VII and figure 2, out of 15 well nourished children of the total 48 measles cases, only 5 children (33.3%) had measles associated complications while out of total 33 malnourished children 25 (75.75%) children had complications and only 8 were free of any complications. It has been observed that malnourished children had higher predilection for measles associated complications as compared to wellnourished measles patient.

Table VIII

Haematological values in Control and study group children of different nutritional grades.

Grade of patient	Control group	Study group			
		Wellnourished	Malnourished		
			Grade I	Grade II	Grade III
Number of cases	20	15	18	10	5
Variables TLC/mm³ Mean±SD	9129.5±1307	8744±1179*	8625±** 3038.5	8654± 1530.27	8070± 3194.5
Hb gm% Mean±SD	13.12±.915	12.73±1.17*	10.27±** 1.22	9.28± 1.02	7.66± 1.16
ESR Mean±SD	15.4±5.80	17.35±18.13*	36.9+++ 15.95	41.8± 15.32	46.2± 13.15

WN- Wellnourished

MN- Malnourished

* Difference was statistically Insignificant when compared to control (P>.05)

** Difference was statistically significant when compared to control and grade III malnourished children compared with grade I MN children (P<.005).

++ Difference was statistically significant when compared to control and other groups of malnourished children (P<.005).

As shown in Table VIII no statistically significant difference was observed in TLC in study group children when compared with control, wellnourished and malnourished measles children and when compared between malnourished children themselves. The Hb gm% in control group was 13.12±.915, while in study group the values were found to be decreased with falling nutritional grades. The Hb gm% in study group was 12.73±1.17, 10.25±1.22, 9.28±1.02 and 7.6±1.16 respectively in wellnourished, grade I malnourished, grade II malnourished and grade III malnourished children respectively. There was a statistically significant difference in Hb gm% values in malnourished measles children when compared with control, no statistically significant difference has been observed in well nourished children when compared with control.The ESR values were found to be significantly higher in all grades of malnourished children when compared with control. The values in measles children were 17-35±18.13, 36.9±15.96, 41.8±15.3 and 46.2±13.15 respectively. The ESR values in control group was 15.45±5.80. There was a statistically significant difference in wellnourished measles children and malnourished measles children, no significant difference in ESR values has been observed between wellnourished children and control group.

Table IX

Immunological values in control group children according to age

Age group (Year)	No.of cases	T Cell	Absolute T cell count	B cell count	Absolute B cell count	DNCB Response (%)
∠1	3	57.3±* 4.15	.2567± 491.5	20.3±* 6.87	927.6±* 164.4	100
1-2	4	58.7±* 4.15	2463± 116	22.2± 3.22	933.2±* 119.3	100
2-4	7	59±** 6.05	2094±** 226.1	22.7±* 8.17	859±** 56.17	85.7
4-12	6	61.3±* 7.04	2259± 286.8	21±* 9.35	914.8±* 184.6	85.3

* No statistically significant difference among different age groups.

** Statistically significant difference when children of below 1 year and 1-2 year group were compared to 2-4 year age group.

As depicted above in Table IX these was no statistically significant difference in T cell count among different age groups of control children. The T cell count in different age groups (viz. ∠1 year, 1-2 year, 2-4 year and 4-12 year) was 57.3±4.15, 58.7±4.15, 59.0±6.05 and 61.3±7.04 and T cell number was 2567±491.5, 2463±11.6, 2094±226.13 and 2259±286.8 when absolute counts were compared mutually there was a statistically significant difference between infants (∠1 year children) and 2-4 year age group and also between 1-2 year and 2-4 year age group. No statistically significant difference has been observed

between other age groups. There was no statistically significant difference in B cell count and absolute number when compared mutually. The observed relative B cell count was 20.3±6.87, 22.2±3.22, 22.7±8.17 and 21±9.35. Absolute B cell counts was 927.6±164.4, 933.2± 119.3, 859.4±56.17 and 914.6±184.6 respectively. DNCB reactivity was 100%, 100%, 85.7% and 85.5% in four different age group of control.

Table X

Immunological values according to sex of control children

Sex	No.of cases	T cell Mean±SD	Absolute T cell count	B cell count	Absolute B cell count	DNCB Response (%)
Male	13	60.48± 5.26	2214± 790	22.7± 6.37	786.3± 437	92.3
Female	7	56.1± 7.03	2199± 417.77	20.7± 11.7	918.14± 1622	85.7
Statistical difference		P7.05 NS	P7.05 NS	P7.05 NS	P7.05 NS	

NS- Not Significant.

Table X shows the immunological parameters like T cell, B cell count, absolute counts and DNCB response in different age group of control group children. No statistically significant difference has been noticed

in above parameters between male and female children. T cell count was 60.48±5.26 and 56.1±7.03 in male and female children respectively. Absolute T cell count was 2214±790 and 2199±417.77 in male and females, B cell count was 22.9±6.37 and 20.7±11.22 in male and female children respectively. The absolute count was 786.3±437 and 918.14±62.9. Response to DNCB has been found to be (92.5%) positive in male and (85.7%) positive in female children.

Table XI

Lymphocyte variation in study group children and in control

Group	Control group	Study group			
		Wellnourished	Malnourished		
			Grade I	Grade II	Grade III
No. of cases	20	15	18	10	5
Lymphocyte count Mean±SD	42.2±3.89	40.3±8.08*	32.8±7.15**	31.7±6.78	28.4**±5.57
Absolute lymphocyte count	3877±465.3	3630±725.9*	2764±73.15**	2694±484.87	2152.4±** 2.40

WN- Wellnourished

MN- Malnourished

* Difference was statistically insignificant($P > .05$)

** Difference was statistically significant($P < .005$) (when MN groups were compared with WN and control children and also when grade I MN children compared with grade III MN children).

As depicted above in table XI and figure 3 the lymphocyte count has been observed to be less with severity of nutritional status. There was significant lymphopenia in malnourished measles children as compared to wellnourished measles children and control. Significant difference was also observed in lymphocyte count between grade I and grade III malnutrition children. Similarly absolute lymphocyte number has also been observed to follow the same pattern. The relative lymphocyte count in control group children was 42.2±3.87 and the absolute count was 3877±465.3. The lymphocyte number in wellnourished measles children was 40.3±8.08 and in malnourished measles children the numbers were 32.8±7.13, 31.7±6.78 and 26.4±5.57 respectively. The absolute lymphocyte count in measles children were 3630±725.9, 2764±73.15, 2694±484, and 2152±240 respectively. The difference in wellnourished measles children and malnourished measles children was statistically significant.

Table XII

Distribution of T cells in study and control groups

Group	Control group	Study group			
		Wellnourished	Malnourished		
			Grade I	Grade II	Grade III
Number of children	20	15	18	10	5
T cell count Mean±SD	59.4±8.27	35±5.57	29.8± 4.06	29.3±** 5.41	24.8±** 3.15
Statistical difference when compared to control		P<.005 Significant	P<.005 Significant	P<.005 Significant	P<.005 Significant
Absolute T cell count Mean±SD	2293.4± 178.13	1233± 2842	820.7± 214.5	802±** 252.2	598.6** ±148.2
Statistical difference when compared to control		P<.005 Significant	P<.005 Significant	P<.005 Significant	P<.005 Significant

** Difference was statistically Significant.

-when compared between WN measles children and grade II and grade III MN children.

-when compared between grade I MN and grade III MN children.

WN- Wellnourished

MN- Malnourished

As shown in the Table XII and figure 4 there has been observed a significant fall in T cell number in malnourished measles children, when compared to wellnourished measles patient and control. The T cell number in control was 59.4±8.27 and the absolute count was 2293.4±278.13. In measles children the relative T cell number was 35±3.57, 29.8±4.06, 29.3±5.41 and 24.8±3.19 respectively. The absolute count were 1233±282.2, 820.7±214.5, 802±252.2 and 598.6±148.2 respectively. The difference in relative T cell count in wellnourished and malnourished measles children was statistically significant when compared to control.

Table XIII

Distribution of B cell in study group and in control group

Group	Control group	Study group			
		Wellnourished	Malnourished		
			Grade I	Grade II	Grade III
Number of children	20	15	18	10	5
B cell count Mean±SD	23.5±3.62	23.4±4.51	22.7±3	20.8±1.84**	19±2.74**
Statistical difference when compared to control		NS	NS	P<.05 S	P<.05 S
Absolute B cell count Mean±SD	899±294.1	834.1±205	827±176	662±133.9**	613±146**
Statistical difference when compared to control		NS	NS	P<.05 S	P<.05 S

S-Significant NS-Not Significant

** Difference was statistically Significant(P<.05) when compared between grade II MN children and control, when compared between grade III MN children and control.

As depicted above in Table XIII and figure 5 the humoral immunity has not been found to be significantly affected in measles except in severely malnourished children. In whom the difference was statistically significant when compared to control and wellnourished measles children. The relative B cell number in control children was 23.5±3.62 and the absolute count was 899±294.1, the relative count in measles children were 23.4±4.51, 22.7±3, 20.0±1.89 and 19±2.14. The absolute B cell numbers were 854.1±205, 827±176, 662±133.9 and 613±146 restively.

Table XIV

Immunological values in acute and convalescent phase of measles

Variable	Mean±SD		P-Value acute Vs. convalescent
	Patient acute phase (n=8)	Patient convalescent phase (n=8)	
TLC/mm^3 (Control) (No.20)	8288±1395.14 9129.5±1307	9928.7±2253.25	(P<.05) Statistically Significant
Absolute lymphocyte count (Control)	2858.6±905.8 3877±465.3	4173.3±3621.4	P>0.05 (NS)
T lymphocyte count (Control)	30.2±5.93 59.4±8.27	41.8±4.25	P<.005 Statistically Significant
Absolute T cell count (Control)	1022.75±307.43 2293.4±178.13	2156±439.6	Statistically Significant
B cell count (Control)	21.75±2.95 23.5±3.62	24.6±3.80	Statistically insignificant
Absolute B cell count (Control)	685.5±290.2 899±294.1	842.5±335.65	Statistically insignificant

Table XIV illustrates the different immunological parameters like TLC, Absolute lymphocyte count, T cell count, Absolute T cell count, B cell count and absolute B cell count in acute and convalescent phase of measles. Eight of the patients were followed up in the study and their immunological responses were evaluated again at 6 week. A significant statistical difference were observed in convalescent phase. The total leucocyte count in acute phase of eight measles patient were 8288±139.514 and in convalescent phase 9928.7±2253.25. The absolute count were 2858.6±905.8 and 4173.3±3621.4 respectively. A significant difference was observed in relative T cell number which was 30.2±5.93 is acute phase while in convalescent phase it was 41.8±4.25. However there was no statistically significant difference in absolute T cell number. There was no significant difference in B cell number in acute Vs. convalescent phases. They were 21.75±2.95 and 24.6±9.80 respectively. When the immunological parameters in acute and convalescent phase of measles were compared to control group children. A statistically significant difference has been observed in TLC absolute lymphocyte count, T cell count, absolute T cell count, when compared to control, no significant difference in B cell count was observed when compared with control.

Table XV

DNCB skin reactivity in study group and in control

Groups	Control group	Study group			
		Wellnourished	Malnourished		
			Grade I	Grade II	Grade III
No.of cases	20	15	18	10	5
DNCB response negative	2(10%)	3(20%)	4(22.2%)	3(30%)	3(60%)
1+	1(5%)	2(13.3%)	4(22.2%)	2(20%)	2(40%)
2+	4(20%)	4(26.6%)	8(44.4%)	4(40%)	
3+	13(65%)	6(40%)	2(11.1%)	1(10%)	
Total+ve cases Number(%)	18/20(90%)	12/15(80%)	14/18(77.7%)	7/10(70%)	2/5(40%)

As illustrated above in table XV the DNCB skin reaction has been found to be in direct proportion of nutritional status severe the degree of malnutrition more negative the DNCB reaction. The DNCB response in different nutritional grades of study group children was 80%(WN) 77.7(MN group 1), 70%(MN group 2) and 40% respectively. While in control DNCB skin test was positive in 90% of cases.

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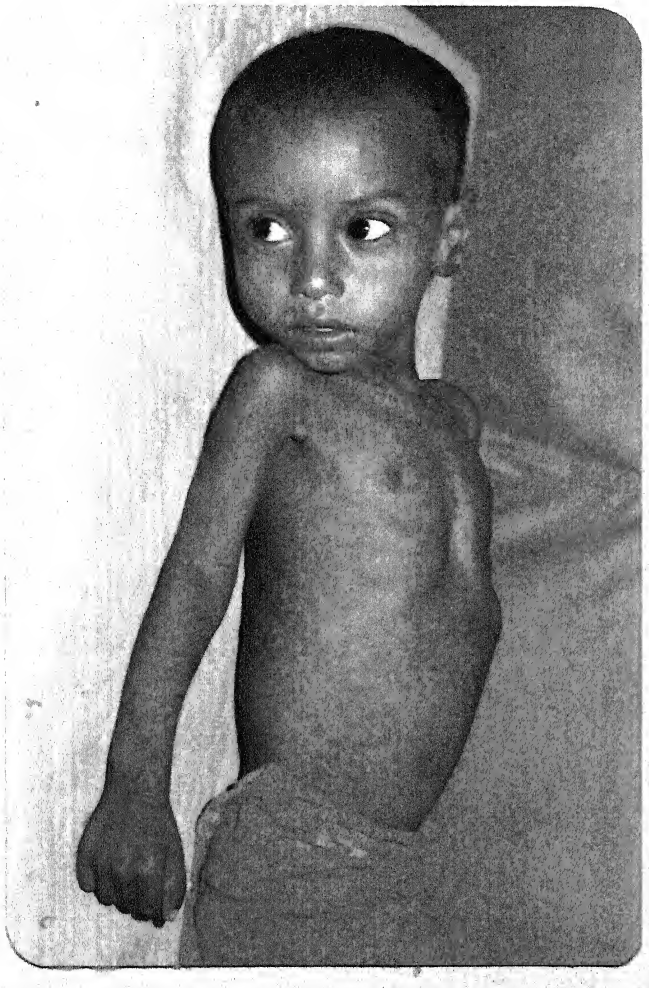

DISCUSSION

DISCUSSION

The present study was conducted on 48 children of measles of different nutritional grades. They were classified into well nourished and malnourished group depending on Harward weight/age standard. The study group children were age matched with control group children. Both the study and control group children were investigated for immunological profile by T cell count and DNCB skin test (for cellular immunity) and B cell count (for humoral immunity). Before evaluating the immunological status of these children total leucocyte count (TLC), Differential Leucocyte Count (DLC), Haemoglobin (Hb) and Erythrocyte Sedimentation Rate (ESR) estimation was done of every case. While no significant variation was observed in Total Leucocyte Count (TLC) when values compared between study and control group children, Hb gm% values were found to be in direct proportion of nutritional status and ESR values were observed to be in inverse proportion of nutritional status. The high ESR values in malnourished study group children could be explained on the basis of severity of anemia in malnourished children as well as due to intercurrent infections observed frequently during measles.

IMMUNOLOGICAL STATUS OF HEALTHY CHILDREN ACCORDING TO AGE AND SEX

No significant variation was observed in immunological parameters eg. T cell, B cell and absolute counts in control group children of either sex and among different age groups. Beside this no significant difference in responsiveness to DNCB contact sensitization was observed in either sex. Our observation are well supported by previous studies. Wybran et al (1973) and Meiburger (1976) found no difference in resetting values in healthy adults and infants, which could be attributed to age or sex. However fleischer et al (1975) found T cell percentage significantly, lower in the younger than elder children and adults. B cell percentage was significantly higher in younger children than elder children and adults. However because the young children had absolute lymphocytosis, so the absolute number of T cell was higher in younger children than elder children and adults.

Twenty (42) of our study group children belonged to age group between 2-4 years, and out of total 48 cases 36 (75%) were below 4 years of age. The age of study group children ranged between 8 months

and 12 years. No case was seen below 6 months of age. As revealed by previous studies (Morley D 1969), measles is a disease of younger children in developing countries, while in developed countries like United Kingdom and Europe the disease occurs in children above 5 years of age. The maximum age incidence in this study was same as observed by earlier workers (Pereira and Benzamin 1972, Krishnamurthy and Ananthraman 1974, Morley D 1976 and Bhasharam P et al 1984).

IMMUNOLOGICAL PARAMETERS IN CONTROL AND STUDY GROUP

Insignificant change in lymphocyte count percentage and absolute count was observed in well nourished measles children when compared to control group. However lymphocyte percentage and absolute lymphocyte count was significantly lowered in malnourished children with measles as compared to control. There could be two reasons of lymphopenia in malnourished children first, as suggested by Smythe et al (1972) that a preexisting state of malnutrition might produce diminution of cell mediated immunity via reduction in the population of thymus dependent lymphocytes and second being immunosuppression due to the atrophy of thymus (White and Boyd 1973) which is frequent in children dying

from measles. Joseph et al have attributed this immunosuppression replication of measles virus in T and B lymphocytes in the perepheral blood which may lead to destruction of these cells thus contributing to deplition of lymphocytes. It has been suggested (Whittle et al 1980, Doosetor et al 1977) that lymphocytes of children with malnutrition are abnormally susceptible to infection by measles virus. The infection is followed by a normal cellular and humeral immune response. As a result, severe damage occurs and this response generates immunosuppressive factors in the patients plasma thus making the child susceptible to secondary infection.

T-lymphocytes in control and study group

As evident from previous studies (Smythe et al 1971, White and Boyd 1973, Whittle and Doosetor 1980) the various causes contributing to T-lymphocytopenia have been found to be preexisting state of malnutrition, atrophy of thymus and replication of measles virus in peripheral blood lymphocytes and all contributing to depression of cell mediated immune response. In this study the T-lymphocyte count as well as absolute count were found to be significantly reduced following acute measles during 0-7 days of appearance of rash in children who

were belonging to malnourished study group. The measles children of wellnourished group had no significant change in lymphocyte, but had a significant difference in T-cell count when compared to control.

Previous studies (Bhaskaram et al 1986, Ron Dagan et al 1987 have also indicated a significant depression in T-lymphocyte subsets following acute measles. A significant degree of immunosuppression in cellular immunity (T-lymphocyte subset) have been observed in malnourished children as compared to well nourished measles children.

Correlation between T-cell subset and degree of malnutrition

It is clear from above account that severity of malnutrition has direct correlationship with depression of cell mediated immunity. As postulated earlier by Smythe et al (1971), a preexisting state of malnutrition in measles can demolish the cellular immune response via reduction in the population of thymus dependent lymphocytes. Contrary to this and our study, Whittle has demonstrated that in malnourished children, although peripheral blood mononuclear cells (PBM) support a higher replication of measles virus, their cellular immunity does not seem to differ from that in well nourished

children (Dossettor J et al 1977). Studies by Bhaskaram et al (1984) have indicated that there is an equal degree of immunosuppresion in both malnourished as well as well nourished children. However their subsequent studies have revealed a significant depression in circulating T-lymphocytes, in severely malnourished children compared to those of other nutritional grades. Recent studies by Ron Dagon et al (1987) have also shown that there was a more impressive depression in T-cell count than the B cell in malnourished children.

B-lymphocyte in control and study group children

Previous studies by different workers (Whittle H C and Dossettor 1978, Ron Dagon et al 1987) have found no significant change in B cell profile following acute measles. However Coovadia et al (1978) have observed a significant depression in both T and B cell number following acute measles during first few days of the rash. Our studies support the observations of Whittle and Dossetor (1978) and Ron Dagon et al (1987). No significant depression in B cell percentage and absolute count has been observed in this study except for moderate to severly malnourished children who had a relative depression in B lymphocyte percentage and absolute count

of our control group children responded to DNCB. It has been observed that the negative DNCB reaction was related to degree of malnutrition. Severe the malnutrition higher the negative reaction to DNCB. The depressed DNCB reactivity was significant in grade II and grade III malnutrition children with measles (70% and 40% respectively). DNCB response in well nourished measles children in our study is comparable to previous study (Whittle H C and Bradley Moore 1973), who found DNCB response positive in 94% of measles children as against 80% positive in our study group children. Study comparing DNCB skin contact response in malnourished and well nourished measles children was not done to our knowledge.

Correlation between DNCB reactivity and T cell count

The skin contact sensitization with DNCB is a test of the ability to process a new antigen and initiate, denove a (cell Mediated Immune) CMI response. As evident from table XI and XIV, DNCB reactivity had direct correlation to T cell percentage in all the 48 measles children. 33 malnourished children who had severe T-lymphocytopenia responded poorly to DNCB skin contact sensitization (77.7%, 70% and 40% respectively). While rest 15 well nourished measles children had a significantly better responsiveness to DNCB (80%) when compared to control

(90%). Our observations support the study of Sanjeev Rao et al (1981) who found more negative reaction to DNCB contact sensitization in malnourished children when compared to control. It has been suggested that severe the degree of malnutrition more negative the response to DNCB. As postulated earlier by Smythe et al (1971), a preexisting state of malnutrition in measles can demolish the cell mediated immune response via reduction in the population of T-lymphocytes. Therefore one can infer that DNCB response is significantly depressed in measles when it is accompanied with malnutrition. Many studies (Smythe et al 1971, Chandra et al 1972, Betsy C Bang 1975 and David McMurry 1981), highlighted the DNCB skin reaction as a test of cellular immunity in malnourished children and found that they did not responded well to DNCB.

Correlation of measles, malnutrition and complications

Many studies by different workers have highlighted the synergistic impact of measles and malnutrition on the host. There is little doubt that severe malnutrition has profound influence on disease resistance mediated by impairement of immune function. Different reasons have postulated that measles is severe in malnourished children owing to defect in the formation of activated lymphocytes. It has been suggested (Whittle et al 1980, Dossetor et al 1977), that lymphocytes of children with malnutrition are abnormally susceptible to infection by measles virus, the impaired cellular immune response allows wide spread infection

with virus. Eventually when the immune response is raised a large number of infected cells are destroyed resulting in extensive allergic damage. The cause of freequent secondary infection was not discussed, but it is generally assumed to be a consequence of the cellular anergy that follows measles. It is clear from above account that a preexisting state of malnutrition might produce diminution of cell mediated immunity via rediation in the population of T-lymphocyte (Smythe et al 1971), attributed to thymic dysplasia (Roberts P F 1975) and due to abnormal susceptiblity of lymphocytes to infection by measles virus which could predispose to severe or fatal measles.

Our study provide strong evidence for a reduction in cell mediated immune function in children who were under weight for the age. However our results are in contradiction with other workers Whittle et al (1980) and Bhaskaram et al (1984), who reported equal degree of immunosuppression in both well nourished and malnourished children, but they have noted a significantly lowered cellular immune response (T-lymphocyte percentage) in severely malnourished children in their further studies (Bhaskaram et al 1986). In view of these observations, including ours, it could be deduced that a preexisting

state of malnutrition demolish the cellular immune response in measles and further aggravates the complications and flare up the latent infections.

Our study was a prospective investigation of the development of impaired cellular immune response in different grades of malnourished measles children. While others (Bhaskaram et al 1986, Ron Dagon et al 1987), have not highlighted it in children of different nutrition grades including different age groups of children.

When age incidence of complications was studied, it was particularly observed that majority of the study group children aged below 4 year were found to have maximum complications (66%). The older children (4-12 years) had complications in 30% of cases only. Our observations are well in accord with previous studies (Sunderavelli et al 1979). Dorai Rajan and Krishnamurthy (1979) have also observed maximum complications in children below 3 years. Coovadia et al (1978) observed a high morbidity and mortality in infants during measles. The poor nutrition was found to be a major contributing factor.

While studying the incidence of various complications this study has observed respiratory complications in

maximum number of cases (69.2%) followed by gastrointestinal (23%) and then neurological (7.7%). The data are fairly in accord with previous studies (Silhar and Maru, 1958, Ghosh and Dhatt 1961, Desai and Churshah 1967) which also indicated highest incidence of respiratiory complications in 87.3%, 86.6% and 37% of cases respectively. When correlation was established between nutritional status and complications it was noted that 68% (33) of our study group children were malnourished and 79% of these children were found to have measles associated complications. A significant difference in morbidity was observed in well nourished children with measles which numbered 15 (32%) and complications were found in 33% cases only.

Being hospital based study this does not reflect the true incidence of complications in measles, and is bound to differ from field studies. It can be presumed that serious and sick children were brought to seek medical advise in the hospital, therefore we found increased concentration of complicated cases in our study. However our observations are supported by reports from previous studies (Ghosh and Dhatt 1961, Krishnamurthy 1979), who also observed a greater number of complications among malnourished hospitalized children with measles.

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APPENDIX

CASE SHEET

Topic- Immune Reactivity on measles.

Case No.- MRD No.-

Name- Age/Sex-

Father's Name- D.O.A.-

Address- D.O.D.-

Diagnosis:

Immunization:

Polio-

DPT-

BCG-

Measles-

Present Illness:

Symptoms- Duration-

Fever-

Cough-

Rash-

Diarrhoea-

Any Other-

Past History:

Pertusis-

Chronic diarrhoea-

Worm Infestation-

Tuberculosis-

Asthmatic bronchitis-

Others-

Family History:

Kochs-

Chronic Kilners-

General Examination:

Pulse/Heart Rate-

Resp.Rate-

Tempreture-

Blood Pressure-

Weight-

Height/Length

Head Circumference-

Oedema

Jaundice-

Anaemia-

Cyanosis-

Clubbing-

Hydration-

Lymphadeno Pathy-

Cervical-

Axillary-

Inguinal-

Systemic Exam:

Resp.System:

Auscultatory findings

CVS-

CNS-

Investigations-

Blood-

ESR-

DLC-

Urine 1.Albumin
2.Sugar

Hb-

ESR-

X-ray Chest-

T. Cell Count (E Rosette)

B. Cell Count (EAC Rosette)

DNCB Skin test:

		Response	
Sensitizing	48 hrs.	14th day.	21st day.

Challange dose- I

II

Signature of Co-guide

Signature of Guide